

CLAIMS

1. An immunoassay comprising the steps of:
 - (a) mixing a whole blood sample with sensitized
5 insoluble carrier particles smaller than erythrocytes to cause an immune agglutination reaction;
 - (b) introducing the resulting immune agglutination reaction mixture including agglutinated particles and unagglutinated particles to a flow cell, irradiating the particles
10 passing through the flow cell with laser light, and detecting scattered lights generated thereby;
 - (c) setting a threshold value for distinguishing unagglutinated particles from agglutinated particles and a threshold value for distinguishing the agglutinated particles
15 from blood cells with regard to intensity of the scattered light; and
 - (d) distinguishing and counting the unagglutinated particles, the agglutinated particles and the blood cells from the scattered lights detected in the step (b), in reference to the
20 threshold values set in the step (c).
2. An immunoassay according to claim 1 further comprising (e) calculating a degree of agglutination from the number of the unagglutinated particles and the number of the
25 agglutinated particles, converting the degree of agglutination into the concentration of an antigen or antibody in the whole blood sample using a calibration line produced beforehand.
3. An immunoassay according to claim 2 further
30 comprising (f) correcting the concentration of the antigen or antibody according to the number of the blood cells.
4. An immunoassay according to claim 3, wherein the

correction is made by use of the following formula:

$$C = CO / (1 - B / A) ,$$

wherein C is a corrected value, CO is the concentration of the antigen or antibody in the whole blood sample, B is the number of blood cells and A is a constant.

5. An immunoassay according to claim 1 or 2 further comprising (g) obtaining a mean corpuscular volume (MCV) in the whole blood sample, wherein the concentration of the antigen or antibody is corrected according to the MCV measurement and the number of blood cells.

6. An immunoassay according to claim 5, wherein the mean corpuscular volume (MCV) is obtained from the scattered lights detected in the step (b), in reference to the threshold values set in the step (c).

7. An immunoassay according to claim 5, wherein correction according to the MCV measurement and the number of blood cells is made by use of the following formula:

$$C = CO / \{1 - (B/A) \times (MCV / D)\},$$

wherein C, CO, A and B are the same as defined above, MCV is the MCV measurement of the sample and D is a constant.

8. An immunoassay according to Claim 1, wherein the scattered light is forward scattered light.

9. An immunoassay according to Claim 1, wherein the size of the insoluble carrier particles is $0.1 \mu m$ to $1.0 \mu m$.

10. An immunoassay according to Claim 1, wherein, in the step (a), the temperature is from 20 to 50 °C and the time is from 15 seconds to 20 minutes.

11. An immunoassay according to Claim 1 which is conducted by use of an apparatus for a counting immunoassay utilizing a principle of flow cytometry.

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12. An immunoassay according to claim 1, in which the sample to be assayed is a plurality of whole blood samples, among which a serum sample is involved.

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13. An immunoassay apparatus comprising:

a reaction part for mixing a whole blood sample with sensitized insoluble carrier particles smaller than erythrocytes to cause an immune agglutination reaction;

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a dispensing mechanism for introducing the resulting immune agglutination reaction mixture including agglutinated particles and unagglutinated particles to a flow cell,

a laser for irradiating the particles passing through the flow cell with laser light, and

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a photo acceptance unit for detecting scattered light generated thereby,

signal processing means for converting a light signal to an electrical signal,

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data processing means for setting a threshold value for distinguishing unagglutinated particles from agglutinated particles and a threshold value for distinguishing the agglutinated particles from blood cells with regard to signal based on intensity of the scattered light; and for distinguishing and counting the unagglutinated particles, the agglutinated particles and the blood cells according to the set threshold values.

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14. An immunoassay apparatus according to Claim 13, further comprising:

calculating means for calculating a degree of agglutination from the number of the unagglutinated particles and the number of the agglutinated particles, converting the degree of agglutination into the concentration of an antigen or antibody in the whole

- 5 blood sample using a calibration line produced beforehand; and correcting the concentration of the antigen or antibody according to the number of the blood cells.